TISSUE ENGINEERING AND BIOPRINTING

DISCUSSIONS AND HOW OUR PROJECT CONTRIBUTES TO THIS APPLICATION

Our concept of DNA programmed cell assembly can aid the construction of complex tissues with single cell level precision.

The current need and design of tissue engineering technologies

Research into synthetic organs, and tissue engineering has been prompted by the rapid growth in patients on a waiting list for organ donation.

"Every year around a thousand people die waiting for a transplant. However, only 4% of people regularly give blood, and only a third of us have joined the Organ Donor Register." - NHS Website

Due to the chronic shortage of donors, the ability to synthesise replicas of organs is a necessity to prevent organ failure. The construction of synthetic organs has also been beneficial due to fewer issues of organ rejection, or the prolonged need for immunosuppressant medication.

Bio-printing is the process of fabricating cells for organs, which need to be patterned to match the compartmentalised structure found within human organs. This is being applied to regenerative medicine to address the need for tissues and organs suitable for transplantation. Making tissues is also important for understanding disease using them for both drugs testing and disease models.

Some researchers have taken advantage of 3D printing techniques in order to print vascular networks of multiple cell types as shown in Fig 1.. These can be produced making a gel matrix which can be used to construct multilayer tissues[1].

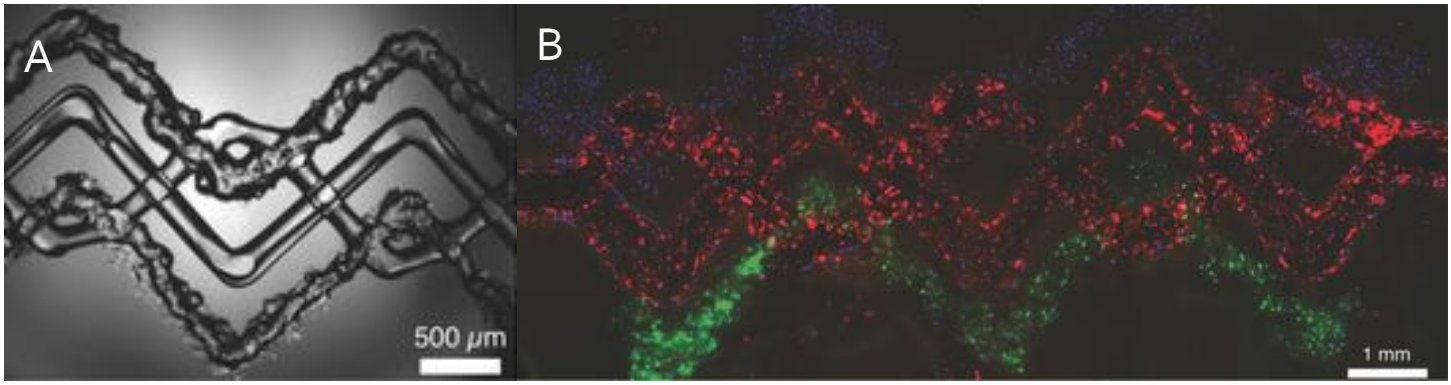

Figure 1: Images from Kolesky 2014

A) Microscopy image of a tissue construct printed in 3D
B) Bo The 3D printed tissue using 3 cells types each labelled in a fluorescent colour

Opinions on tissue engineering – advice on how our method could be utilised

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There is a need for getting cells into the right position for tissues, as everything has an order. If we can predict the necessary structure and repeat in vitro, there's a possibility to make a functional tissue where different cells can interact because they are in the right place.

Photon lithography is used to create order within tissue as it allows polymers to crosslink. It Uses a high resolution laser to pinpoint and crosslink gelatin to make shapes [2].

If the 3D structure is designed well enough with DNA origami it may influence tissue engineering. If you can get a few layers of cells then you can have one of top of the other and can mass produce say adipose with the other layers. Another use of your method is to have production steps for the different parts - blood vessels, endothelial cells. By breaking it into parts you could then assemble these together.

Another potential approach to ordering tissues would be to culture these with pathogens. The idea of placing mammalian cells, and then have a layer of pathogens, to make pathogen models and look at the response to drug testing.

The role of our project in tissue engineering

The ability to organise cells using DNA programmed cell assembly may be another method that can contribute to tissue engineering by tackling the issue of structure. Constructing tissue requires heterogeneous features to be assembled at a single cell level. We have modelled possibilities of how a 3D structure could possibly be assembled which can be viewed on our modelling page. Our work found an inherent problem of scaling up and making complex shapes. The technique, similar to lithography, would not make a replica of a tissue but could be used to produce a patch of tissue. This would be achieved depositing stratified layers, with each subsequent layer needing to be smaller than or equal to the previous.

Our method has been done in bacteria, *E.coli,* yet the purpose of synthetic biology allows parts to be expressed in different organisms. Some researcher groups have considered assembling mammalian tissue with a similar technique of DNA programmed assembly. This system doesn't use a DNA-zinc finger system, but instead coats cells in single stranded DNA which bind together forming double stranded DNA and bringing together cells in close contact [3]. However the general idea of using a system of two parts that come together, in our case a receptor and a ligand is very similar. This demonstrates how the research does have a possibility of being used for tissue engineering. Our technique could also have potential within tissue engineering as another research group, from whose research we based our zinc fingers on, expressed the some of the same zinc fingers in mammalian cells and made them bind to double stranded DNA [4].

If our system could be applied universally in to both mammalian and human cells this would behighly desirable. It means that pathogen models can be produced by constructing a structure with designed layers of mammalian cells, coated in bacterial pathogens. Currently scientists investigating how pathogens colonise the gut use microfluidics to separate cells but allow them to share the same environment [5].

[1] Kolesky, David B., et al. "3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs." *Advanced Materials* 26.19 (2014): 3124-3130.

[2] Lin, Hang, et al. "Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture." *Biomaterials* 34.2 (2013): 331-339.

[3] Todhunter, Michael E., et al. "Programmed synthesis of three-dimensional tissues." *Nature methods* (2015).

[4] Mali, Prashant, et al. "Barcoding cells using cell-surface programmable DNA-binding domains." *Nature methods* 10.5 (2013): 403-406.

[5] Kim, Jeongyun, Manjunath Hegde, and Arul Jayaraman. "Microfluidic co-culture of epithelial cells and bacteria for investigating soluble signal-mediated interactions." Journal of visualized experiments: JoVE 38 (2010).